Aquaxan™

# Technical report

## Aquaxan™ HD algal meal use in aquaculture diets: *Enhancing nutritional performance and pigmentation*

(TR.2102.001)

The benefits of using Aquaxan HD algal meal in aquaculture diets are reviewed:

- ***Aquaxan HD algal meal is prepared from the alga Haematococcus pluvialis.***
- ***It is an excellent natural source of algal micronutrients, especially astaxanthin, a unique carotenoid pigment used to improve nutritional performance and pigmentation properties of diets for salmon, trout, shrimp, red seabream, and other marine or tropical aquatic species.***
- ***The natural astaxanthin stereoisomer found in Aquaxan HD is the same as that found in the natural food of the aquaculture species targeted, and is the same as the dominant astaxanthin stereoisomer found in their flesh, unlike the synthetic form.***
- ***Astaxanthin in Aquaxan HD algal meal has a high bioefficacy.***
- ***Trials have shown that Aquaxan HD algal meal has excellent pigmentation properties comparable to synthetic astaxanthin, and that feeding Aquaxan HD algal meal resulted in higher weight gain.***
- ***Studies indicate that algal astaxanthin has a higher bio-efficacy than synthetic astaxanthin, especially when used in larval and postlarval shrimp feeds, resulting in improved survival.***
- ***Astaxanthin has been attributed vitamin-like properties in fish. Its functions include pro-vitamin A activity, pigmentation, photoresponse and communication, antioxidant, reproduction and development, and a role in immune response mechanisms.***
- ***Requirements for astaxanthin are reviewed and recommendations on usage of Aquaxan HD algae meal are provided***

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## 1. Aquaxan HD algae meal

- Is an algal meal prepared from ***Haematococcus pluvialis***. *Haematococcus* spp. are ubiquitous green algae (Chlorophyceae) in the family Volvocales. They are encountered thoughout the world and naturally occur in fresh and brackish waters[1,2,3,4]. When environmental conditions become inhospitable (e. g., drying out of pools), *Haematococcus* cells start reddening, accumulating lipids and **astaxanthin for protection againt photooxidation and other oxidative mechanisms**, while entering a resting phase [1,2,3,4] .

- Is produced by cell-breaking *Haematococcus* algae and gentle drying at low temperature to **ensure minimum degradation and maximum bioavailability of astaxanthin and other micro-nutrients**.

- Is stabilised with the antioxidant ethoxyquin, which ensures a **satisfactory stability when stored at 20°C** or below[6] and comes with a **guaranteed total astaxanthin content**[5].

- Is an excellent source of natural **algal micronutrients, including essential amino acids and polyunsaturated fatty acids** to enhance nutritional performance of aquaculture diets.

- Is particularly rich in **astaxanthin**, a natural **red pigment** that improves pigmentation **of salmon, trout, red seabream and shrimp**, but also has other very important biological functions including **pro-vitamin A activity, communication and photoresponse, protection of lipids against oxidation, protection against light and photooxidation, reproduction, larval development and growth, immune response and health.**

- Contains primarily **esterified astaxanthin**, a **more stable** form than free astaxanthin in nature[8] although **highly bioavailable**[9].

- Is mainly composed of the **3*S*,3'*S*** astaxanthin enantiomer[10], the **same predominant astaxanthin isomer found in wild salmon**[11], while in other sources such as yeast or synthetic astaxanthin, other isomer forms predominate[12].

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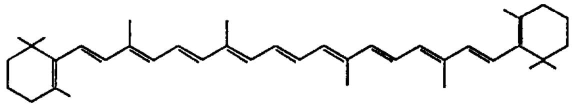
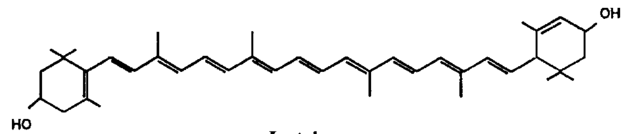
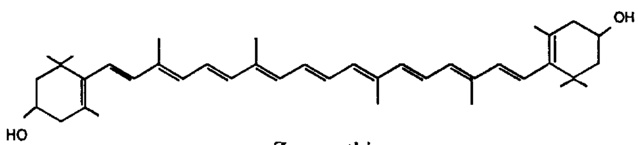
## Fig. 1. Structure of selected carotenoids



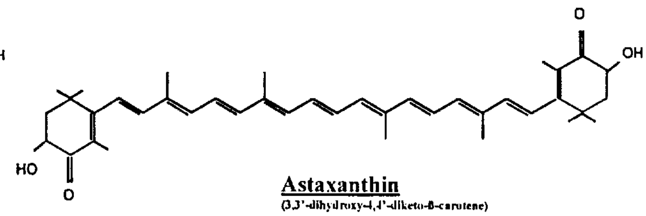
Beta-carotene



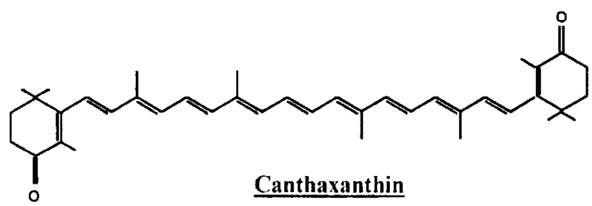

Lutein




Zeaxanthin

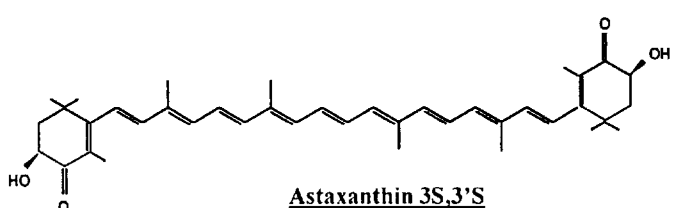



Astaxanthin
(3,3'-dihydroxy-4,4'-diketo-β-carotene)

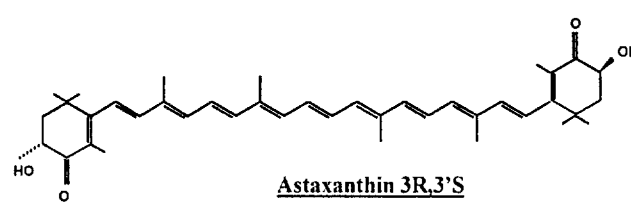



Canthaxanthin

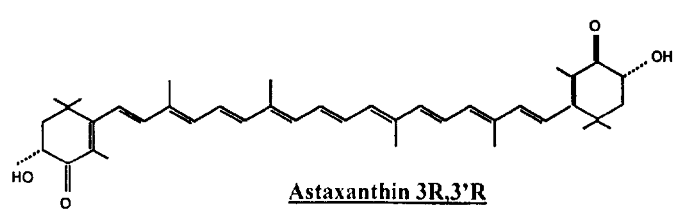
## Fig. 2. Astaxanthin enantiomers




Astaxanthin 3S,3'S
(3,3'-dihydroxy-4,4'-diketo-β-carotene)




Astaxanthin 3R,3'S




Astaxanthin 3R,3'R

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## 2. Astaxanthin natural occurrence.

- **Astaxanthin** is the **main carotenoid pigment** found in aquatic animals[13]. It can be found at significant levels in important aquaculture products such as **salmon, trout, red seabream, shrimp, lobster, and fish eggs**[7,14].

- **Astaxanthin cannot be synthesised** by animals and must be provided in the diet as is the case with other carotenoids [7,14]. While salmonids are unable to convert other dietary carotenoids into astaxanthin[7], some species such as crustaceans have a limited capacity to convert closely related dietary carotenoids into astaxanthin, although feeding astaxanthin directly to shrimp rather than precursors results in better pigmentation due to conversion inefficiencies[14,53].

- **Form and level of deposition of astaxanthin differ between tissues**: esterified astaxanthin predominates in the skin, teguments, and eggs, while free astaxanthin is the main form in the flesh, serum and other internal organs of salmon[7]. In shrimp, esterified astaxanthin predominates, except in the ovaries and eggs[17,18]. In red seabream, mostly esterified astaxanthin is found in the skin [17,18] . The more stable esterified form is believed to be an adaptive feature to be able to store astaxanthin in tissues without excessive oxidation[8].

- **Esterified 3S,3'S astaxanthin,** the main astaxanthin enantiomer **in Aquaxan HD algae meal** is the **dominant astaxanthin form in natural foods/preys** of aquaculture species[11]. This 3*S*,3'*S* astaxanthin enantiomer is the same as the main enantiomer found in the **flesh of wild salmon**[11]. Salmonids seem to be unable to convert the 3*R*,3'*S* enantiomer in synthetic astaxanthin to the natural 3*S*,3'*S* form[11]. Fillets from farmed salmon fed synthetic astaxanthin will have characteristically high levels of the 3*R*,3'*S* form and can therefore be easily distinguished by analytical means from the wild salmon[11].

**Table 1. Main forms of astaxanthin in tissues of important aquaculture species**

| *Tissues* / *Species* | Skin | Flesh | Digestive gland | Ovaries | Serum | Eggs |
|---|---|---|---|---|---|---|
| Salmonids [7] | Esterified | Free | Free | Free | Free | Esterified |
| Shrimp [15,16] | Esterified | Esterified | Esterified | Free | | Free |
| Red Seabream[17,18] | Esterified | N.A. | N.A. | N.A. | N.A. | N.A. |

N.A.: Not available

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**Table 2. Form and level of astaxanthin in selected important aquaculture species and potential astaxanthin sources**

**Form and level of astaxanthin in selected important aquaculture species and potential asta**

| | Astaxanthin | | | |
|---|---|---|---|---|
| | **Content (mg/kg)** | **Free/esterified** | **Main isomer** | **Reference** |
| <u>Aquaculture species</u> | | | | |
| Sockeye salmon | 26-37 | Free,esterified** | 3*S*,3'*S* | 11,7 |
| Coho salmon | 9-21 | Free,esterified** | 3*S*,3'*S* | 11,7 |
| Chum salmon | 3-8 | Free,esterified** | 3*S*,3'*S* | 11,7 |
| Chinook salmon | 8-9 | Free,esterified** | 3*S*,3'*S* | 11,7 |
| Pink salmon | 4-6 | Free,esterified** | 3*S*,3'*S* | 11,7 |
| Atlantic salmon | 3-11 | Free,esterified** | 3*S*,3'*S* | 11,7 |
| Rainbow trout | 1-3 | Free,esterified** | 3*S*,3'*S* | 7 |
| salmon eggs | 0-14 | esterified*** | N.A. | 19,20 |
| Red seabream | 2-14 | esterified*** | N.A. | 17,18 |
| Red seabream eggs | 3-8 | N.A. | N.A. | 20 |
| *Peneaus monodon* | 10-150 | Esterified,free** | 3*S*,3'*S* | 16 |
| Lobster | | Esterified,free** | N.A.* | 12, 37 |
| <u>Astaxanthin sources</u> | | | | |
| Copepods | 39-84 | esterified*** | N.A.* | 7 |
| Krill | 46-130 | esterified*** | 3*R*,3'*R* | 7 |
| Krill oil | 727 | esterified*** | 3*R*,3'*R* | 7 |
| Crayfish meal | 137 | esterified*** | N.A.* | 7 |
| Arctic shrimp | 1160 | esterified*** | 3*S*,3'*S* | 7 |
| Yeast (*Pfaffia rhodozyma*) | 30-800 | esterified*** | 3*R*,3'*R* | 7 |
| Synthetic astaxanthin | 80,000 | free | 3*R*,3'*S* | 7 |
| ***Haematococcus pluvialis*** | **10,000-30,000** | **esterified*** ** | **3*S*,3'*S*** | **9** |

* Crustaceans are believed to have mostly the 3*S*,3'*S* form, Krill might be the exception.

** depending on tissues, free or esterified astaxanthin may be found

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## 3. Bioefficacy of algal astaxanthin.

- In **Red Seabream** *(Chrysophrys major)*, pigmentation efficacy of the **esterified form of natural astaxanthin** was reported **superior to synthetic free astaxanthin** [21,18].

- Recent work conducted in Thailand also showed **superior bioefficacy of astaxanthin from *Haematococcus* over the synthetic form**, in larval and post-larval shrimp (*Penaeus monodon*) diets, leading to higher survival [22]. Survival of shrimp zoea fed diets supplemented with 200 ppm algal astaxanthin was found to be 3 times higher than those fed diets supplemented with the same amount of synthetic astaxanthin. In the case of mysis larvae and post-larvae, the algal astaxanthin diets resulted in 20% and 18% improved survival over the synthetic astaxanthin diets.

- In **salmonids**, our trials (Fig. 3, Fig. 4) have shown that properly cell-broken and gently dried algal meal from ***Haematococcus***, resulted **in pigmentation and astaxanthin deposition in the flesh, comparable to that obtained from synthetic astaxanthin, when trout were** fed diets supplemented with equivalent levels of these two pigment sources[23]. Portion-size trout (*Oncorynchus mykiss*) fed the algal astaxanthin (Aquaxan HD algal meal) for 90 days, grew to a higher final weight than those fed the synthetic form, suggesting a **superior bioefficacy**, similar to what has been observed with shrimp. An earlier study found that feeding a diet supplemented with astaxanthin from partly-broken *Haematococcus* cells (60% broken cells), resulted in astaxanthin deposition levels in the flesh of trout which were 260% of the level achieved with non-broken cells and 58% of synthetic astaxanthin[24]. This earlier study had concluded that the lower pigmentation efficacy of algal astaxanthin in trout, compared to the synthetic form, could be attributed to two possible causes: insufficient breakage of cell wall and/or lower absorption rate due to the need for fish to hydrolyse the esterified form into free astaxanthin before it can be absorbed and transferred into the blood and organs[24,25]. Assuming the linearity of the pigmentation efficacy and the percentage of broken cells, an extrapolation of those earlier results would have indicated that diets prepared with 100% broken cells would most likely have resulted in astaxanthin deposition very similar to those obtained with the synthetic astaxanthin. Because of the toughness of their cell walls, *Haematococcus* cysts are difficult to rupture, and since the astaxanthin is enclosed inside those cell walls, it is very important to maximise breakage of the cells, without destruction of the astaxanthin. The processing of Aquaxan HD algal meal has been designed to achieve a thorough rupture of the cell walls to ensure the best bio-availability of algal astaxanthin, while minimising losses. Our very good pigmentation efficacy results and earlier work in Japan which found equivalent pigmentation efficacy between synthetic free astaxanthin and esterified natural astaxanthin in coho salmon *(Oncorhynchus kisutch)*[26], concur with studies in red seabream and indicate that **esterified algal astaxanthin and** synthetic free astaxanthin have similar pigmentation efficacy in salmon or trout.

## Pigmentation and growth results with trout fed algal or synthetic astaxanthin

Fig 3
Astaxanthin deposition in muscle of trout fed 10, 25 or 40 ppm astaxanthin

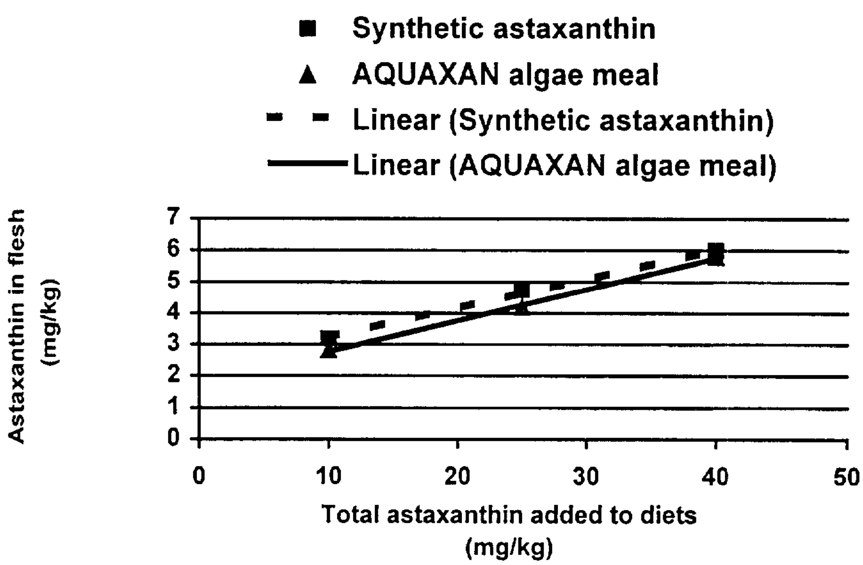
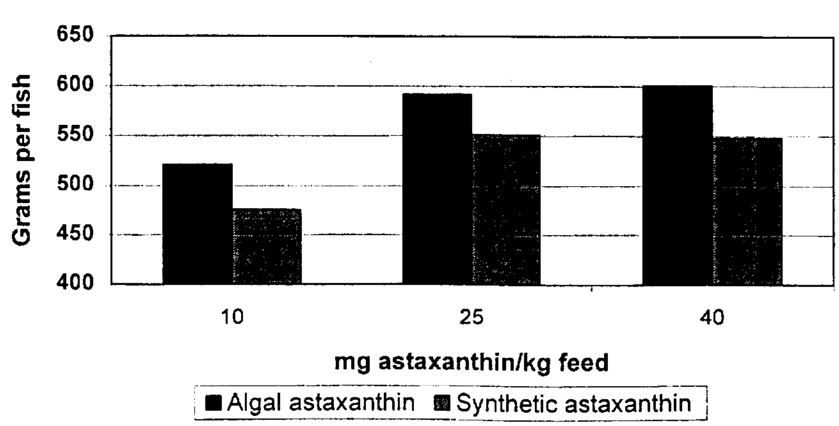



Fig.4.

Final weight (g/pc) of fish fed diets supplemented with 10, 25 or 40 ppm algal or synthetic astaxanthin



## 4. Functions of astaxanthin:

The main functions of astaxanthin in aquatic species include:

- Pro-vitamin A activity
- Pigmentation, photo response and communication
- Antioxidant properties
- Reproduction and development
- Immune response mechanisms

### 4.1. Pro-vitamin A activity – role in vision

- Retinoids, including Vitamin A, are well known for their role as vision pigments. In fish, vitamin A has been shown to be an essential vitamin, with deficiency leading to xerophthalmia and cataracts, while supplementation in the diet prevented these deficiency symptoms and promoted growth[27].
- Astaxanthin, as with other carotenoids including beta-carotene, has been reported to play a role as precursor of vitamin A in salmon *(Salmo salar)* and trout *(Oncorhynchus mykiss)* [28,29], tilapia (*Tilapia nilotica*) [30], guppies (*Lebistes reticulatus*) and platies (*Xiphophorus variatus*) [31].
- It should be noted that in the deep sea stomatoid fish *Malacosteus niger*, astaxaxanthin is a tapetal pigment, believed to function as a diffuse reflector which increases visual sensitivity[32].

### 4.2. Astaxanthin's role in photoresponse, communication and behaviour

- Fish are known to change coloration in response to changes in lighting and background, during reproductive behaviour or when excited, as both a way of communicating and protecting themselves[33].
- Carotenoid pigments, accumulating and migrating within chromatophores and xanthophores spread out in the tegument of fish, are responsible for these colorations and their changes. It has been suggested that the bright colors of male salmons during reproductive period, resulting from astaxanthin accumulation, is a secondary sexual characteristic that may influence the behaviour and be a condition of the success of the reproductive process[33].
- This role in communication and behaviour is believed to be a major function of carotenoids in the animal world[34].

### 4.3. Antioxidant properties of astaxanthin: the SUPER VITAMIN E.

- Astaxanthin has been shown to be an excellent natural antioxidant[13,35,36,36,38]. Astaxanthin is very active against singlet oxygen ($^1O_2$), hydroxyl radicals ($^{\cdot}OH$), and organic free radicals, and was found to be more active than other carotenoids (zeaxanthin, beta-carotene, canthaxanthin) or alpha-tocopherol on those free radical species. Indeed, when compared to vitamin E, the in-vitro activity of astaxanthin was found to be 15 times higher on free radicals, and 100 times on singlet oxygen [13].

- *In-vitro* studies have shown astaxanthin to be the most effective natural antioxidant to protect linolenic acid, a polyunsaturated fatty acid (PUFA) from peroxidation[13]. PUFAs are considered critical components of cell membranes of marine fish and shrimp, who have elevated dietary requirements for essential PUFAs. Those PUFAs are very sensitive to oxidation due to their double bonds.
- Astaxanthin is also believed to protect tissues from photo-oxidation by UV light, e. g., in salmon swimming in shallow waters, or in salmonid eggs. [7]
- Astaxanthin has been attributed a protective effect on some essential vitamins. Feeding trials have shown that tissues of Atlantic salmon (*Salmo salar*) fed astaxanthin-supplemented diets had 2 to 20 times higher levels of physiologically active antioxidant vitamins (retinol, alpha-tocopherol, ascorbic acid) supporting an antioxidant sparing property of astaxanthin on those vitamins[39].

**4.4. Role of astaxanthin in reproduction and development.**

- Carotenoids, and more specifically astaxanthin, have long been attributed an important role in reproduction of shrimp and fish[7,14].
- It has been noted that astaxanthin deposited in the flesh is mobilised and redeposited in ovaries and the skin during the reproductive cycle of salmonids[14].
- Salmon eggs contain high levels of lipids, specifically polyunsaturated fatty acids (PUFAs), which are critical to the success of reproduction and larval development. It is assumed that astaxanthin plays an important protective role for these PUFAs as a natural *in-situ* antioxidant[14].
- In red seabream (*Chrysophrys major*), astaxanthin has been found to improve buoyancy and other egg quality parameters- and production of larvae when broodstock were fed diets containing astaxanthin [20]
- Feeding astaxanthin to yellow tail broodstock resulted in improved egg quality [40].
- Recent work has demonstrated that astaxanthin was essential for high survival and rapid growth of newly-hatched salmon fry and juveniles [41,42].

**4.5. Effects of astaxanthin on health and immunology**

Astaxanthin has been reported to improve both specific and non-specific immune response mechanisms in fish:

- In salmonids, astaxanthin improved survival of Atlantic salmon submitted to an *Aeromonas salmonicida* challenge, and has been demonstrated to be essential for the survival of salmon fry [43,46].
- *In-vitro* experiments with trout phagocytes have demonstrated the immuno-stimulatory effect of astaxanthin, which is believed to protect the cell membranes of the phagocytes from oxidation [44].
- Higher astaxanthin levels have been found in phagocytic cells of trout such as phagocytes, macrophages and neutrophils, indicating a greater need for autoprotection against toxic oxidative by-products [41].
- Supplementation of astaxanthin in salmonid diets has been shown to affect *in-vivo* all non-specific immune response parameters tested [45].
- Those results corroborate a large number of studies which have demonstrated the positive effect of astaxanthin in specific and non-specific immune response

mechanisms in mammals [46,47,48,49,50,51]. Astaxanthin has been shown to have anticarcinogenic effects in mice[48,51,63,66], to stimulate formation of antibody-forming cells in the spleen of sheep[46,47], to enhance *in-vitro* production of T-cell-dependent antigen in normal strains of mice and possibly antibody production[47].

- Two freshwater fish, *Oreochromis nilotica* and *Colisa labiosa,* fed astaxanthin at 32 or 71 mg/kg, displayed improved histology of the liver, a critical organ which plays an essential role in immune response mechanisms in fish [52]. Astaxanthin supplementation has resulted in improved growth of tilapia [53].
- Finally, the sparing effect of astaxanthin on other essential vitamins with immune response functions may also have an indirect positive effect on the health and immune response of fish[39].

In shrimp, astaxanthin has also been shown to improve survival and immune response.

- In Kuruma shrimp (*P. japonicus*), 50 to 100 ppm dietatry astaxanthin has been shown to improve survival and growth [54,55,57].
- In Tiger prawns (*P. monodon*), 100 to 200 ppm dietary astaxanthin has been shown to improve resistance to bacterial and viral infections[56], while only 50 ppm is sufficient to prevent the blue-shrimp syndrome [16].
- More recently, dietary astaxanthin was shown to improve survival of larval and post-larval shrimp (*P. monodon*), with algal astaxanthin showing a superior effect over the synthetic form[22].

## 4. Requirements for astaxanthin – recommended supplemental levels

- The increased mortality and reduced growth observed in salmon fed astaxanthin-free diets support the assumption of a vitamin-like property of astaxanthin and of an essential requirement for it[28].
- The minimum requirement for optimal growth and survival of Atlantic salmon fry has been determined to be 5.3 ppm astaxanthin[41]. However to ensure optimal pigmentation, much higher levels are recommended: as high as 50 to 70 mg astaxanthin per kg of feed is common practice in the industry.
- In shrimp, although essentiality of carotenoids seems to be widely accepted [15,58,60], exact requirements have not been determined. Crustaceans, including penaeid shrimp, are able to convert other carotenoids to astaxanthin, although this conversion may be slow or inefficient[58]. Indeed, astaxanthin has been reported to be more effective than beta-carotene and other carotenoids at improving survival[54] and astaxanthin deposition and pigmentation [54,57,58] in peneaid shrimp.
- Dietary levels of 25 to 50 ppm dietary astaxanthin are recommended to correct the blue-shell syndrome of *Penaeus monodon* [16].
- In Kuruma shrimp, *Penaeus japonicus*, it has been found that astaxanthin deposition increased to a maximum 38 mg/kg in the flesh, 85 mg/kg in the head and 54 mg/kg in the shell, when fed up to 200 ppm astaxanthin, with no additional pigmentation efficacy if feeding higher levels than 100 ppm.

- On the other hand, levels as high as 100 to 400 ppm have been recommended for improved survival, immune response and resistance to disease in shrimp [59,60,61].
- In red seabream, no specific requirements have been determined, but the industry frequently adds up 25 to 50 ppm astaxanthin to commercial diets, since poorly pigmented red seabream have significantly lower marketing acceptance leading to lower selling prices. .
- Astaxanthin is also added to larval and starter diets, often at significantly higher levels than in grower diets. In the case of shrimp larval and postlarval diets, recent work shows that 200 ppm algal astaxanthin is an adequate level and maximises resistance to stress[22].

Table 3 Recommendations on astaxanthin supplemental levels in aquaculture diets.

| Suggested supplemental astaxanthin levels (mg/kg feed): | | | | |
|---|---|---|---|---|
| | **Starter/larval diets** | | **Grower diets** | |
| | **Low** | **High** | **Low** | **High** |
| **Salmonids** | **30** | **50** | **40** | **80** |
| **Red seabream** | **30** | **60** | **30** | **60** |
| **Shrimp** | **100** | **200** | **10** | **50** |

Table 4: Conversion table: algal meal inclusion/supplemental astaxanthin targeted

| Supplemental astaxanthin target level (ppm) | Astaxanthin level in Aquaxan HD algae meal | | | | |
|---|---|---|---|---|---|
| | 1.0% | 1.5% | 2.0% | 2.5% | 3.0% |
| | Inclusion in feed (kg/Ton feed) | | | | |
| 1 | 0.10 | 0.07 | 0.05 | 0.04 | 0.03 |
| 5 | 0.50 | 0.33 | 0.25 | 0.20 | 0.17 |
| 10 | 1.00 | 0.67 | 0.50 | 0.40 | 0.33 |
| 15 | 1.50 | 1.00 | 0.75 | 0.60 | 0.50 |
| 20 | 2.00 | 1.33 | 1.00 | 0.80 | 0.67 |
| 30 | 3.00 | 2.00 | 1.50 | 1.20 | 1.00 |
| 40 | 4.00 | 2.67 | 2.00 | 1.60 | 1.33 |
| 50 | 5.00 | 3.33 | 2.50 | 2.00 | 1.67 |
| 60 | 6.00 | 4.00 | 3.00 | 2.40 | 2.00 |
| 70 | 7.00 | 4.67 | 3.50 | 2.80 | 2.33 |
| 80 | 8.00 | 5.33 | 4.00 | 3.20 | 2.67 |
| 90 | 9.00 | 6.00 | 4.50 | 3.60 | 3.00 |
| 100 | 10.00 | 6.67 | 5.00 | 4.00 | 3.33 |
| 120 | 12.00 | 8.00 | 6.00 | 4.80 | 4.00 |
| 140 | 14.00 | 9.33 | 7.00 | 5.60 | 4.67 |
| 160 | 16.00 | 10.67 | 8.00 | 6.40 | 5.33 |
| 180 | 18.00 | 12.00 | 9.00 | 7.20 | 6.00 |
| 200 | 20.00 | 13.33 | 10.00 | 8.00 | 6.67 |
| 250 | 25.00 | 16.67 | 12.50 | 10.00 | 8.33 |
| 300 | 30.00 | 20.00 | 15.00 | 12.00 | 10.00 |

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*Technical report*

# Astaxanthin in nature

(TR.3001.001)

- ***Astaxanthin is the main carotenoid pigment found in aquatic animals.***
- ***Studies suggest that it can be 10 times more powerful than other carotenoids and more than 100 times than vitamin E, as a biological antioxidant.***
- ***It plays a role in many essential metabolic functions in animals: protection against oxidation and UV-light, vision, immune response, pigmentation and communication, reproduction, and development.***
- ***In some species it has been attributed vitamin-like properties and is believed to be essential to normal growth and survival.***
- ***The micro-alga Haematococcus pluvialis holds nature's record of astaxanthin concentration, at more than 3% of dry biomass.***
- ***The main astaxanthin stereoisomer found in Haematococcus is the same as that found in wild salmon. The main form of astaxanthin in Haematococcus is the esterified form, which is also found in several aquatic species. It is the more stable natural form.***

**What is Astaxanthin?**

**Astaxanthin** is the main **carotenoid** pigment found in aquatic animals.[1] This red-orange pigment is closely related to other well-known carotenoids (Fig. 1) such as beta-carotene or lutein, but has a stronger antioxidant activity (10 times higher than beta-carotene)[1]. Studies suggest that astaxanthin can be more than 100 times more effective as antioxidant than vitamin E.[7] In many of the aquatic animals in which it is found, astaxanthin has a number of essential biological functions, including protection against oxidation of essential polyunsaturated fatty acids, protection against UV-light effects, pro-vitamin A activity and vision, immune response, pigmentation, communication, reproductive behaviour, and improved reproduction.[2] In species such as salmon or shrimp, astaxanthin is considered essential to normal growth and survival, and has been attributed vitamin-like properties.[2] Some of these unique properties have also been found to be effective in mammals[3-7] and **open very promising possibilities for nutraceutical and pharmaceutical applications of astaxanthin in humans.**

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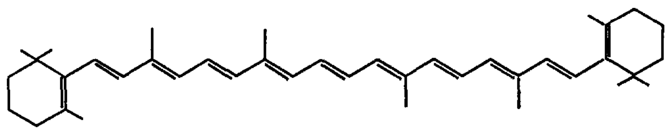
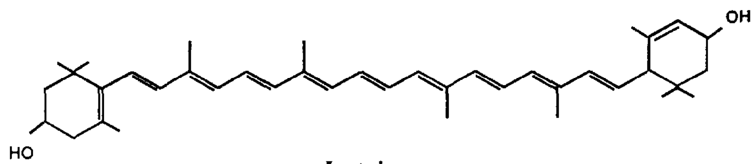
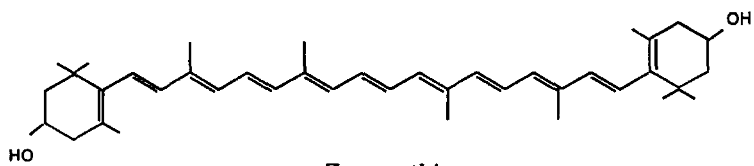
Fig. 1. Structure of selected carotenoids



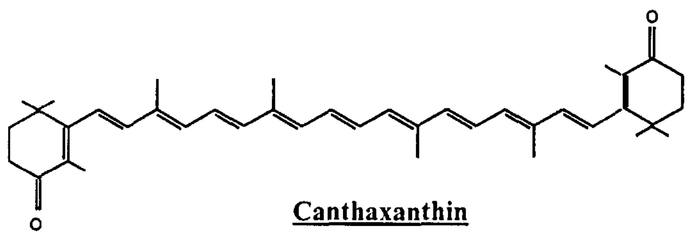
**Beta-carotene**




**Lutein**




**Zeaxanthin**




**Canthaxanthin**


**Astaxanthin**
(3,3'-dihydroxy4,4'-diketoß-carotene)

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**Where Is Astaxanthin Found in Nature?**

Astaxanthin can be found in many of our favorite **seafoods** such as salmon, trout, red seabream, shrimp, lobster, and fish eggs.[2] It is also found in a number of bird species.[8,9] Astaxanthin cannot be synthesised by animals and must be provided in the diet, as is the case with other carotenoids. While fish such as salmon are unable to convert other dietary carotenoids into astaxanthin,[2] some species such as shrimp have a limited capacity to convert closely related dietary carotenoids into astaxanthin, although they benefit strongly from being fed astaxanthin directly.[10] Mammals lack the ability to synthesise astaxanthin, or to convert dietary astaxanthin into vitamin A: unlike beta-carotene, astaxanthin has no pro-vitamin A activity in mammals.[24] Some micro-organisms can be quite rich in astaxanthin. A ubiquitous micro-alga, ***Haematococcus pluvialis*****,** is believed to be **the organism which accumulates the highest levels of astaxanthin in nature**. The function of astaxanthin appears to be to protect the alga from adverse environment changes, such as increased UV-light photoxidation that can occur if the water pools in which it lives dry out.[11-13] *Haematococcus* algae can accumulate as much as 10 to 30 g of astaxanthin per kg of dry biomass. This level is **1,000 to 3,000 fold higher than in salmon fillets!** Some strains have even been observed to accumulate as much as 70 to 80 g of astaxanthin per kg of dry biomass.

**What Forms of Astaxanthin are Found in Nature?**

Form and location of astaxanthin deposition differ between tissues and species (cf. Tables 1 & 2). Esterified astaxanthin predominates in the skin, teguments, and eggs, while free astaxanthin is the main form in the flesh, serum and other internal organs of salmon.[2] In shrimp, esterified astaxanthin predominates, except in the ovaries and eggs.[17,18] In red seabream, mostly esterified astaxanthin is found in the skin.[14,15] The more stable esterified form is believed to be an adaptive feature to be able to store astaxanthin in tissues without excessive oxidation.[1] **Esterified astaxanthin** is the main form found in ***Haematococcus pluvialis***.

Although they have the same chemical composition, 3 main spatial configurations or stereoisomers of the astaxanthin molecule can be found in nature. They are the 3*S*,3'*S*, 3*R*,3'*S*, and 3*R*,3'*R* isomers, characterised by the orientation of the two hydroxyl groups on the molecule (cf. Fig. 2). A **recent study by FDA** concluded that the **3*S*,3'*S* is the main form found in wild Pacific and Atlantic salmon species** and that in order to achieve the same astaxanthin profile as their wild counterparts, farmed salmon should be fed a diet containing the same astaxanthin profile as in the natural diet of wild salmon.[16] The **3*S*,3'*S* isomer is the main form** found in H*aematococcus pluvialis,* while synthetic astaxanthin contains primarily the 3*R*,3'*S* isomer. Salmon appear unable to convert the 3*R*,3'*S* isomer into the more common 3*S*,3'*S* form. In fact, the FDA study clearly showed that farmed salmon could be easily distinguished from the wild salmon because the farmed salmon are fed synthetic astaxanthin and accumulate astaxanthin isomers in the flesh in the same ratio as is found in their diet. This suggests that consumers may prefer to eat farmed salmon fed a natural form of astaxanthin.

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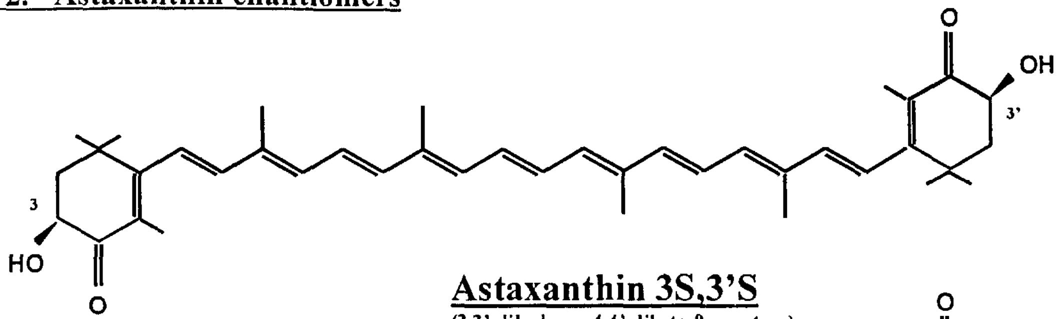
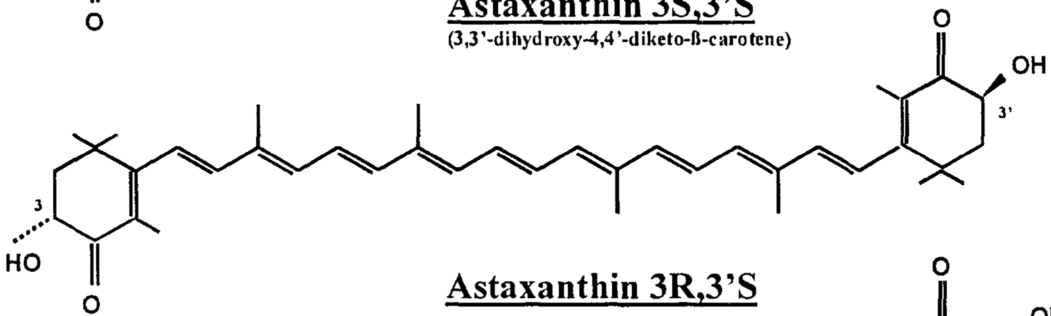
**Fig. 2. Astaxanthin enantiomers**

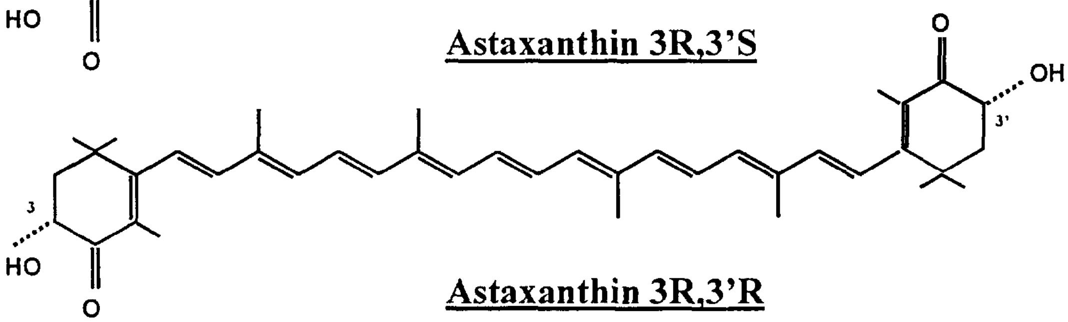



**Astaxanthin 3S,3'S**

(3,3'-dihydroxy-4,4'-diketo-ß-carotene)




**Astaxanthin 3R,3'S**




**Astaxanthin 3R,3'R**

**Table 1. Main forms of astaxanthin in tissues of important aquaculture species**

| Tissues | Skin | Flesh | Digestive gland | Ovaries | Serum | Eggs |
|---|---|---|---|---|---|---|
| Species | | | | | | |
| Salmonids[2] | Esterified | Free | Free | Free | Free | Esterified |
| Shrimp[17,18] | Esterified | Esterified | Free | Free | N.A. | Free |
| Red Seabream[14,15] | Esterified | N.A. | N.A. | N.A. | N.A. | N.A. |
| N.A. : not available | | | | | | |

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## Technical report

# Astaxanthin as an antioxidant: a summary.

(TR.3002.001)

- *Powerful oxidising agents such as free radicals, e..g. hydroxyl and peroxyl radicals, as well as the highly reactive forms of oxygen, such as singlet oxygen, are produced in the body during metabolic processes. They can cause severe damage to cells, affect immune response mechanisms, and have been associated with ageing and a number of pathological conditions including atherogenesis, ischemia-reperfusion injury, infant retinopathy, age-related macular degeneration, and carcinogenesis.*
- *There are two broad classes of biological antioxidants which can counteract those effects: the preventative antioxidants and the radical-scavenging antioxidants. Carotenoids and vitamin E belong to both groups.*
- *Carotenoids can act as quenchers of singlet oxygen and other reactive species, by absorbing the excited energy of singlet oxygen onto the carotenoid chain, leading to the degradation of the carotenoid molecule, but preventing other molecules or tissues from being damaged. They can act also as chain-breaking anti-oxidants and therefore protect lipidic membranes from rapid degradation.*
- *Astaxanthin antioxidant properties as a quencher of singlet oxygen and scavenger of free radicals, and its ability to protect lipids from peroxidation, have been largely demonstrated. Studies indicate astaxanthin antioxidant properties to be superior by up to 10-fold, when compared to other carotenoids, and by more than 100 fold, when compared to vitamin E.*
- *Astaxanthin antioxidant properties are believed to be at the core of most of its potential benefits in human health. In mammals, unlike beta-carotene, astaxanthin lacks pro-vitamin A activity. As a result, astaxanthin antioxidant properties cannot be diverted into vitamin A synthesis. In addition, astaxanthin has the ability to cross the blood-brain barrier, unlike beta-carotene. It has therefore the ability to directly exert its antioxidant properties in those organs.*

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## Technical report

### 1. What is biological oxidation?

Oxidation is the chemical process by which an atom, molecule or ion robs another of one or more of its electrons. Chemicals exhibiting this tendency for stealing electrons are referred to as oxidising agents. Perhaps the most familiar oxidising agent is oxygen itself. We can see many examples of oxygen doing its electron-stealing in our everyday lives: the browning of an apple, the rusting of an iron nail, the slow fading of blue jeans. When a material is oxidised, its chemical structure is altered, often irreversibly. In biological systems, such as the human body, a number of powerful oxidising agents can cause damage to cells. Electron-stealing molecules known as free radicals (hydroxyls and peroxyl radicals, etc...), as well as the highly reactive forms of oxygen, such as singlet oxygen, are produced in the body during various normal metabolic reactions and processes. Physiological stress, air pollution, tobacco smoke, exposure to chemicals, and exposure to ultraviolet (UV) light or other forms of ionising radiation can all enhance the production of these unwanted oxidising agents[2]. Phagocytes involved in the immune response against micro-organisms can also generate an excess of free radicals to aid their defensive degradation of the invader. Within cells, free radicals can damage DNA, proteins, and lipid membranes. Such damage has been linked to aging[3,4] and a number of pathological conditions including atherogenesis[5,6], ischemia-reperfusion injury[7,8], infant retinopathy[9], age-related macular degeneration[10], and carcinogenesis[11,12,13].

### 1. What are biological antioxidants?

Biological antioxidants are defined as "compounds that protect biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidations" [14]. There are two broad classes of biological antioxidants: the preventative antioxidants and the radical-scavenging antioxidants. Preventative antioxidants, such as catalase and superoxide dismutase, suppress the formation of free radicals. Radical-scavenging antioxidants, such as the flavinoid compounds and vitamin C, serve to "mop up" excess free radicals[15]. Vitamin E and the carotenoids are very important biological antioxidants that act in both preventative and radical-scavenging roles.

### 3. Carotenoids , powerful natural antioxidants

Carotenoids are a class of natural lipid-soluble pigments found principally in plants, algae and photosynthetic bacteria, where they play a critical role in photosynthesis. They also occur in some non-photosynthetic bacteria, yeast and mold, where they may carry out a protective function against damage by light and oxygen. Although animals appear to be incapable of synthesising carotenoids, many animals incorporate carotenoids from their diet. Within animals, carotenoids provide bright coloration, serve as antioxidants, and can be a precursor of vitamin A[16,17]. Carotenoids are responsible for many of the red, orange and yellow hues of plant leaves, fruits and flowers, as well as the colour of some birds, insects, fish and crustaceans. Some familiar examples of carotenoid coloration are the oranges of carrots and citrus fruits, the reds of peppers and tomatoes, and the pinks of flamingos and salmon[18]. Some 600 different carotenoids are known to occur naturally[16].

Carotenoids can act as potent biological antioxidants, especially as quenchers of singlet oxygen and other reactive species, by absorbing the excited energy of singlet oxygen onto the carotenoid chain, leading to the degradation of the carotenoid molecule, but preventing other molecules or tissues to be damaged[19]. Carotenoids can act also as chain-breaking anti-oxidants: free radicals generated within the body can lead to the degradation of polyunsaturated fatty acids, and create a chain reaction leading to the degradation of lipidic membranes within a short time. Carotenoids help break the chain reaction by donating a hydrogen to the damaging unstable free radical[19].

## 4. Astaxanthin as an antioxidant

Astaxanthin's ability to quench singlet oxygen and scavenge free radicals has been demonstrated by a number of studies[1,20-24]. Astaxanthin showed a very good capability at protecting membranous phospholipids[25] and other lipids[1,24] against peroxidation. One of these studies demonstrated that astaxanthin was best among carotenoids at preventing peroxidation of lipids, with up to 10-times higher anti-oxidant efficacy of astaxanthin over beta-carotene[1], while another one demonstrated a superior capacity of astaxanthin over zeaxanthin, canthaxanthin or beta-carotene at reducing peroxidation of unsaturated fatty acids . Superior singlet oxygen quenching ability of astaxanthin has also been demonstrated over other carotenoids such as beta-carotene (up to 1.7[26,27] to 38[28] times higher, depending on testing conditions) or lutein and zeaxanthin [28]. Another important factor to note is that in humans and other mammals, although this is not the case in most aquatic animals, and unlike beta-carotene and other carotenoids, astaxanthin has no pro-vitamin A activity. It can therefore not be diverted from its main function as an antioxidant to become part of the pro-vitamin A pool. Also, the risk of hyper-vitaminosis with excessive accumulation of vitamin A, is reduced. Finally, unlike beta-carotene, astaxanthin has the ability to cross the blood-brain barrier and therefore directly exert its antioxidant properties in those organs[29].
Astaxanthin has also been compared to a well-known non-carotenoid antioxidant: alpha-tocopherol (Vitamin E) and proved to have a superior singlet oxygen quenching capability (80[26,27] to 550[28] times higher) and at preventing lipid peroxidation[1,20]. Experiments with red blood cells and mitochondria from rats have shown that astaxanthin is 100 to 500 times more effective at inhibiting lipid peroxidation than is vitamin E[1,20]. The results of these in vitro studies were confirmed *in vivo* with rats given dietary supplements of astaxanthin and subjected to oxidising agents, [1,20].

These antioxidant properties are believed to be at the source of most potential benefits of astaxanthin in human health. Those include among others[30]:

- Support of the immune system
- health of the eye and central nervous system
- anti-cancer properties
- protection against UV light damage
- blood cholesterol regulation and prevention of arteriosclerosis and related ailments
- response to bacterial infections
- anti-inflammatory response

**Table 1. Singlet oxygen quenching efficacy of astaxanthin: comparison with selected carotenoids and alpha-tocopherol (adapted from Shimidzu et al., 1996[28])**

| Compounds | Physical quenching rate constant (in-vitro) $k_q \times 10^{-9}$ ($M^{-1}$ $s^{-1}$) (measures singlet oxygen quenching ability) | | | |
|---|---|---|---|---|
| | Substrate 1 (CDCl3/CDOD)(2:1) | | Substrate 2 (CDCl3) | |
| Astaxanthin | 1.8 | (367%) | 2.2 | (100%) |
| Zeaxanthin | 0.12 | (245%) | 1.9 | (82%) |
| Lutein | n.d. | | 0.8 | (41%) |
| Beta-carotene | 0.049 | (100%) | 2.2 | (100%) |
| Alpha-tocopherol | n.d.* | | 0.004 | (0.2%) |

n.d. = not determined

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## *Technical report*

# Astaxanthin and health: a summary.

(TR.3003.001)


- ***Astaxanthin's antioxidant properties as a quencher of singlet oxygen and scavenger of free radicals, and its ability to protect lipids from peroxidation, have been largely demonstrated. Studies indicate astaxanthin antioxidant properties to be superior by up to 10-fold, when compared to other carotenoids, and by more than 100 fold, when compared to vitamin E.***
- ***Astaxanthin antioxidant properties are believed to be at the source of most its potential benefits in human health. Unlike beta-carotene, astaxanthin has no pro-vitamin A activity in mammals.***
- ***Possible role of astaxanthin in the immune response, health of the eye and nervous system, photo-protection, and against cancer, inflammation, infections, or artheriosclerosis, is discussed.***


### 1. Astaxanthin as a general biological antioxidant

Astaxanthin (Ax) has been shown to be a powerful quencher of singlet oxygen activity in in vitro studies (DiMascio et al. 1990; Miki 1991), and is a strong scavenger of oxygen free radicals, at least ten times stronger than beta-carotene (Miki 1991). Experiments with red blood cells and mitochondria from rats have shown that Ax is 100 times more effective at inhibiting lipid peroxidation than is vitamin E (Miki 1991). The results of these in vitro studies were confirmed in vivo with rats given dietary supplements of Ax and subjected to oxidising agents (Miki 1991). The antioxidative properties of Ax have been demonstrated in a number of different biological membranes (Kurashige et al. 1990; Palozza and Krinsky 1992; Oshima et al. 1993; Nakagawa et al. 1997). This anti-oxidant activity is believed to be at the origin of a number of astaxanthin beneficial properties in health.

### 2. Astaxanthin as an anti-cancer agent

Studies of the cancer-preventative properties of Ax have been carried out on rats and mice by Takuji Tanaka and colleagues at the Gifu University School of Medicine. Dietary administration of Ax proved to significantly inhibit carcinogenesis in the mouse urinary bladder (Tanaka et al. 1994), rat oral cavity (Tanaka et al. 1995a), and rat colon (Tanaka et al. 1995b). In addition, Ax has been shown to induce xenobiotic-metabolising enzymes in rat liver, a process which may help prevent carcinogenesis (Gradelet et al. 1996).

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### 3. Astaxanthin for support of the immune system

Ax has been shown to significantly influence immune function in a number of in vitro and in vivo assays using animal models. The majority of this work has been carried out by Harumi Jyonouchi and colleagues at the University of Minnesota. Ax enhances in vitro antibody production by mouse spleen cells stimulated with sheep red blood cells (Jyonouchi et al. 1991), at least in part by exerting actions on T-cells, especially T-helper cells (Jyonouchi et al. 1993). Ax can also partially restore decreased humoral immune responses in old mice (Jyonouchi et al. 1994). These immuno-modulating properties are not related to provitamin-A activity, because Ax, unlike beta-carotene, does not have such activity (Jyonouchi et al. 1991). Studies on human blood cells in vitro have demonstrated enhancement by Ax of immunoglobulin production in response to T-dependent stimuli (Jyonouchi et al. 1995a). Other supporting data on Ax and immune function, including studies on the mechanisms of action involved, may be found in Jyonouchi et al. (1995b), Jyonouchi et al. (1996), Okai & Higashi-Okai (1996), and Tomita et al. (1993).

### 4. Astaxanthin for health of the eye and central nervous system

The possible role of antioxidants in alleviating oxidation stress and other oxidative damages to the eye and the nervous system has been extensively reviewed by Trevithick and Mitton (1999). As one of nature's most effective antioxidants with the ability to cross the blood-brain barrier (Tso and Lam, 1996), astaxanthin's potential benefits for the health of the eye and the nervous system, are very promising. The eye is potentially one of the organs which is the most exposed to oxidation, because it is exposed to air and UV-light as well as being irrigated by a very large number of small capillaries capable of bringing many of the metabolic oxidative residues through the blood. Also the eye contains high levels of poly-unsaturated fatty acids and pigments that are quite sensitive to oxidation (Starostin 1988, Donstov et al. 1999). Recently, a research group demonstrated increased superoxide and peroxide formation following UV irradiation of a lens protein (Linetsky et al. 1996). Photoxidation of the lens proteins have been associated to the development of catarct (Taylor, 1993). Carotenoids found in the human retina, lutein and zeaxanthin are closely related to astaxanthin. There is abundant evidence that certain carotenoids can help protect the retina from oxidative damage (Snodderly 1995). Investigations of the antioxidant effectiveness of astaxanthin in the eye are just beginning, but are already very promissing. A recent study with rats indicates that Ax can be effective at ameliorating retinal injury, and that it is also effective at protecting photoreceptors from degeneration (Tso and Lam 1996). The conclusions of this study were that Ax could be useful for prevention and treatment of neuronal damage associated with age-related macular degeneration, and that it may also be effective at treating ischemic reperfusion injury, Alzheimer's disease, Parkinson's disease, spinal cord injuries, and other types of central nervous system injuries (Tso and Lam 1996). In this study, Ax was found to easily cross the blood-brain barrier (unlike beta-carotene), and did not form crystals in the eye (unlike canthaxanthin; Tso and Lam 1996). These conclusions concur with those of Sokol & Papas (1999) who report encouraging results in the possible use of antioxidants to treat or prevent neurodegenerative diseases such as Alzheimer's disease.

### 5. Astaxanthin as a photo-protectant

Light, especially UV light, can trigger photoxidation mechanisms and produce active oxygen species such as singlet oxygen (Noguchi and Niki, 1999, Mc Vean et al. 1999). Lipids (Dontsov et al. 1999, Guillen-Sans & Guzman-Chozas, 1998), pigments (Ostrovskii, 1987, Starostin et al. 1988), DNA (Dunford et al. 1997), proteins (Taylor 1993) have been reported to be sensitive to photoxidation. Oxidative damage to the eye and skin by UV light have been widely documented (Trevithick and Mitton, 1999, Mc Vean et al,. 1999). The strong antioxidative activities of Ax suggest its potential as a photoprotectant, as indicated by the recent study by Tso and Liam (1996) cited above, indicating lower damage by UV light to the eye of animals fed astaxanthin, although the effects of Ax on mice exposed to UV irradiation have not been conclusive (Savouré et al. 1995; Black 1998). Nevertheless, Ax-containing preparations for prevention of light ageing of skin have been developed (Suzuki et al. 1996a, 1996b).

## 6. Astaxanthin and infections

A recent study suggested that Ax may be effective as a prophylactic and/or therapeutic treatment of *Helicobacter* infections of the mammalian gastrointestinal tract, and an oral preparation has been developed for this purpose (Alejung and Wadstroem 1998).

## 7. Astaxanthin for prevention of arteriosclerosis and related diseases

Ax has been shown in both in vitro experiments and in a study with human subjects to be effective for the prevention of the oxidation of low-density lipoprotein (Miki et al., 1998). This suggests that it could be used as a preventative for arteriosclerosis, coronary artery disease, and ischemic brain damage; a number of astaxanthin-containing health products are under development based on these findings (Miki et al. 1998).

## 8. Astaxanthin in anti-inflammatory preparations

According to recent studies, Ax diesters appear to exert a synergistic effect on anti-inflammatory agents, increasing the effectiveness of aspirin when the two are administered together (Yamashita 1995).

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